

# Induction of slow oscillations by rhythmic acoustic stimulation

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## Abstract

Slow oscillations are electrical potential oscillations with a spectral peak frequency of  $\sim 0.8$  Hz, and hallmark the electroencephalogram during slow-wave sleep. Recent studies have indicated a causal contribution of slow oscillations to the consolidation of memories during slow-wave sleep, raising the question to what extent such oscillations can be induced by external stimulation. Here, we examined whether slow oscillations can be effectively induced by rhythmic acoustic stimulation. Human subjects were examined in three conditions: (i) with tones presented at a rate of 0.8 Hz ('0.8-Hz stimulation'); (ii) with tones presented at a random sequence ('random stimulation'); and (iii) with no tones presented in a control condition ('sham'). Stimulation started during wakefulness before sleep and continued for the first  $\sim 90$  min of sleep. Compared with the other two conditions, 0.8-Hz stimulation significantly delayed sleep onset. However, once sleep was established, 0.8-Hz stimulation significantly increased and entrained endogenous slow oscillation activity. Sleep after the 90-min period of stimulation did not differ between the conditions. Our data show that rhythmic acoustic stimulation can be used to effectively enhance slow oscillation activity. However, the effect depends on the brain state, requiring the presence of stable non-rapid eye movement sleep.

## Introduction

Sleep slow oscillations (SOs) of  $< 1$  Hz hallmark slow-wave sleep (SWS) as they are the largest oscillatory events (amplitude  $> 75\mu V$ ) recorded in the electroencephalogram (EEG). They emerge from highly synchronized cortical neuronal networks undergoing alternations between phases of membrane depolarization together with higher firing activity (up states) and phases of hyperpolarized membrane potentials and neural quiescence (down states; Sanchez-Vives and McCormick, 2000; Steriade et al., 1993). SOs have been likewise observed in diverse animal species and humans (Achermann and Borbely, 1997; Mölle et al., 2002; Rattenborg et al., 2011; Steriade, 2006). A large number of studies have demonstrated different functions of the SO, the most important of which is their role in the consolidation of long-term memory (Diekelmann and Born, 2010; Mölle and Born, 2011), and their role in the homeostatic regulation of synaptic connectivity (Tononi and Cirelli, 2006).

The established functional importance of SOs has attracted growing interest regarding their manipulation in terms of both enhancement and suppression (Landsness et al., 2009; Marshall et al., 2006; Van der Werf et al., 2009). The former requires a possibility to trigger SOs, which has been successfully attempted using transcranial direct current stimulation (tDCS; Marshall et al., 2006), transcranial magnetic stimulation (Massimini et al., 2007) or intracranial electrical stimulation (Vyazovskiy et al., 2009). Rhythmic sensory stimulation, to the best of our knowledge, has not been tested as a tool to induce SOs in humans, although acoustic stimuli are well known to induce K-complexes, which are considered a forerunner of the SO (Cash et al., 2009; De Gennaro et al., 2000; Riedner et al., 2011). Based on this evidence and because of the simplicity of the approach, here we probed the capacity of rhythmic stimulation in the 0.8-Hz SO frequency to induce SOs in the human brain. Of particular interest was whether such regular stimulation had the capability to entrain endogenous SO rhythms to an external drive. Effects were tested while subjects were awake,

transited into sleep and during stable non-rapid eye movement (NonREM) sleep. Of additional interest was the question whether rhythmic 0.8-Hz stimulation would accelerate onset of sleep and SWS. A similar effect was achieved in a previous work by instrumental conditioning of the sensorimotor rhythm (Hoedlmoser et al., 2008). Basically, starting the stimulation already during wakefulness before sleep also enabled a comparison between the effects of the stimulation between wakefulness and sleep, which should reveal clues as to a possible brain state dependence of the stimulation effects.

## Materials and Methods

### Subjects, experimental design and procedures

Ten healthy subjects (seven females, three males; mean age =  $22.3 \pm 1.0$  years; range = 18–26 years) participated in the experiments. All participants were non-smokers, and were not using any medication at the time of the experiment. Prior screening ensured no history of neurological or psychiatric disease. Participants were not allowed to ingest alcohol on the day before experimental nights, and were asked to refrain from caffeine 8 h before the scheduled sleeping time. Moreover, they were instructed to get up at 07:00 hours and not to take a nap during these days. Prior to the experiments, subjects were accustomed to sleeping under laboratory conditions during an adaptation night, including EEG recordings and wearing of headphones (but without any stimulation). The experiment was approved by the ethics committee of the University of Lübeck, and subjects gave written informed consent prior to participation.

Each subject was studied according to a within-subject design on three experimental conditions (0.8-Hz stimulation, random stimulation, and sham), with the respective experimental nights separated by at least 5 days. The order of conditions was balanced across participants. In the 0.8-Hz stimulation condition sound bursts were presented with a constant interstimulus interval (ISI) of 1.25 s, corresponding to a frequency of 0.8 Hz as an approximate to the SO frequency. In the random stimulation condition, sounds occurred randomly, with ISIs ranging from 0.125 to 5 s, excluding intervals between 0.5 and 2 s in order not to overlap with effects of the 0.8-Hz stimulation. Random ISIs were generated such that the average ISI was also 1.25 s in this condition, and that in both stimulation conditions the same total number of sounds ( $n = 4416$ ) was presented (which implies a higher amount of short ISIs during the random stimulation condition). Acoustic stimulation commenced 2 min before lights were turned off (at 23:00 hours). During this 2-min interval subjects lied in bed with eyes open fixating a point at the ceiling. After lights off, subjects were allowed to sleep and acoustic stimulation continued for a further 90 min. The sham control condition comprised pe-

riodic acoustic presentation only within the 2 min prior to lights off. The EEG was continuously recorded throughout the whole night until 07:00 hours, when the participants were awakened.

### Acoustic stimulation

The stimuli were bursts of pink  $1/f$  noise of 50 ms duration, with a 5-ms rising and falling time, respectively. Pink instead of white noise was used because it sounds softer and is therefore more comfortable to hear. Sound volume was measured and calibrated prior to each experimental night using a Voltcraft sound level meter SL-400 (Conrad Electronic SE, Hirschau, Germany) to 60 dB SPL, measured directly at the in-ear headphone. Stimuli were presented binaurally via RP-HJE170 in-ear headphones (Philips, Amsterdam, the Netherlands).

### Sleep EEG recordings and polysomnography

The EEG was recorded with a Neurofax EEG-9200 (Nihon Kohden, Tokyo, Japan) from 19 channels (extended 10–20 system, Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2) referenced to C3 and C4 using an Easycap (Easycap GmbH, Herrsching, Germany) and Ag-AgCl ring electrodes. Impedances were kept  $<5$  k $\Omega$ . Signals were filtered between 0.08 and 120 Hz and offline re-referenced to the averaged signal from mastoid electrodes (M1, M2). Vertical and horizontal eye movements (VEOG, HEOG) as well as electromyogram from the chin (EMG) were obtained for standard polysomnography and for artefact detection. All recordings were sampled at 500 Hz and stored for later offline analyses. Electroencephalogram (at C3 and C4), electrooculogram (EOG) and EMG recordings were used for offline scoring of sleep by two experienced raters who were blinded with regard to the experimental condition. Scoring was done for subsequent 30-s recording epochs according to standard criteria (Rechtschaffen and Kales, 1968). Total sleep time and time spent in the different sleep stages (wake; sleep stages 1–4; SWS, i.e. sum of sleep stages 3 and 4; REM sleep) were determined for the total nights as well as for the 90-min periods of acoustic stimulation (and for corresponding periods of the sham condition). Also, sleep onset latency (first occurrence of stage 1 sleep followed by stage 2 sleep, with reference to lights off) and latency of SWS and REM sleep (with reference to sleep onset) were determined. Prior to scoring, the EEG and both EOG channels were low-pass filtered at 30 Hz, and EMG channels were high-pass filtered at 5 Hz. Stimuli evoking arousals or awakenings (as judged by visual inspection) were marked in order to discard them from averaging analyses.

### EEG spectral analysis

Analyses were performed with Spike2 software version 7 (Cambridge Electronic Design, Cambridge, UK) and Brain Vision Analyser 2 (BrainProducts, Munich, Germany). All

EEG signals were pre-filtered between 0.15 and 30 Hz. Beginning with the 2-min wake interval before lights off, a Fast Fourier Transformation (Hanning window, 16 384 data points) was calculated on a 33-s window that was moved in 10-s steps in time for a total of 32 min. Analysis was limited to the 32-min time interval, as individual sleep courses became highly divergent with ongoing sleep resulting in large interindividual variance. To obtain the time course of the activity in the SO (0.5–1 Hz), slow-wave activity (SWA; 0.5–4 Hz), theta (4–8 Hz), slow (9–12 Hz) and fast spindle (12–15 Hz) bands, for each 33-s window the mean spectral power within the respective frequency band was calculated.

### Auditory-evoked potentials (AEPs)

To assess AEP responses for the two stimulation conditions, the EEG signals were averaged with reference to stimulus onset. For the random stimulation condition, stimuli were additionally divided into ‘overlapping’ and ‘non-overlapping’ stimuli, depending on whether or not the stimulus was separated by more than 1.25 s from the previous and following stimulus. As the non-overlapping category included the lowest absolute number of stimuli, for respective comparisons stimulus subsets of equal size were randomly drawn from the category of overlapping stimuli as well as from the set of stimuli in the 0.8-Hz stimulation condition. Signals were averaged for a 1.2-s window including a 0.1-s pre-stimulus onset baseline. To normalize responses, the average potential during this baseline was set to zero. In the same way, slow and fast spindle activities were averaged with reference to stimulus onset. Prior to this analysis the EEG signal was filtered in the respective frequency bands (9–12 Hz, 12–15 Hz), down-sampled to 100 Hz, and the root mean square signal was determined. The baseline normalization was performed as described for AEPs.

### SO detection and statistical analyses

Analyses of SOs were restricted to periods of SWS during the 90-min stimulation period and during the corresponding period of the sham condition. Offline detection of SOs was performed in all EEG channels according to a custom-made algorithm described previously (Möller et al., 2002). In brief, the EEG was band-pass filtered between 0.15 and 30 Hz, and down-sampled to 100 Hz. For the identification of large SOs a low-pass filter of 3.5 Hz was applied. Then, negative and positive peak potentials were derived from all intervals between consecutive positive-to-negative zero crossings (i.e. one negative and one positive peak between two succeeding positive-to-negative zero crossings). Only intervals with durations of 0.8–2 s (corresponding to a frequency of 0.5–1.25 Hz) were included. A SO was identified as such only if both absolute negative and negative-to-positive peak potentials were larger than 1.5 times the respective average. Averages of original EEG potentials in a 3-s window  $\pm 1.5$  s around the peak of the negative half-wave of all identified SOs were calculated. To examine

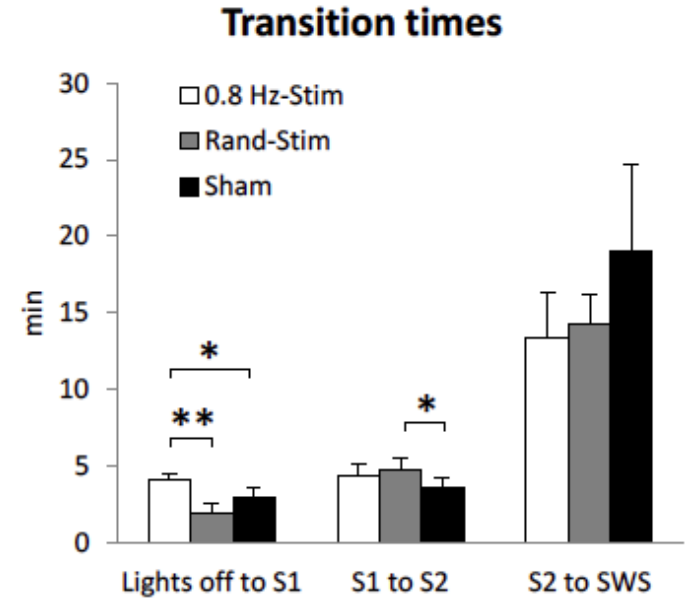
whether rhythmic acoustic stimulation enhanced the occurrence of SOs in trains of several succeeding oscillations, auto-event correlation analyses were performed using 9-s intervals with 4.5-s offset and a bin-size of 0.1 s. The histograms were referenced to the negative half-wave peaks of the SO.

Statistical differences between experimental conditions were assessed using analyses of variances (anova) and paired t-tests. We concentrate here on results from t-tests that are reported only after respective anova indicated significance for the main or interaction effects of interest. A P-level  $< 0.05$  was considered significant.

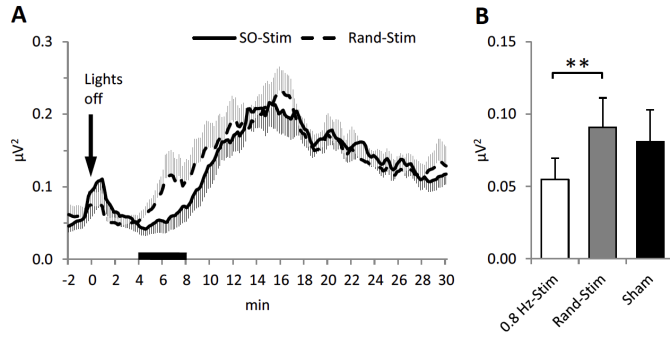
## Results

### Rhythmic 0.8-Hz stimulation delays sleep onset

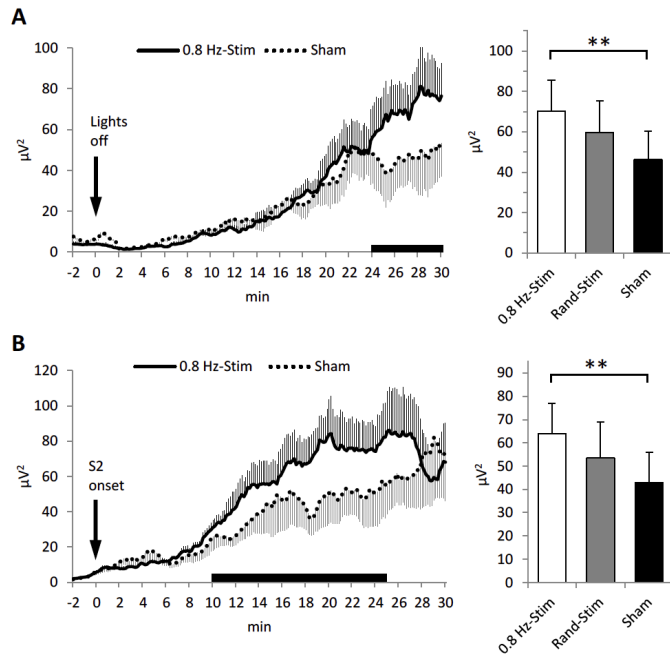
Fig. 1 shows the average time subjects needed to transit between stages, beginning from wakefulness to the first occurrence of stage 1 sleep, from stage 1 to stage 2 sleep, and from stage 2 sleep into SWS. Contrary to expectations, in the 0.8-Hz stimulation condition subjects needed significantly more time to reach stage 1 compared with both the random stimulation ( $P < 0.01$ ) and the sham ( $P < 0.05$ ) condition, indicating a delayed sleep onset. The transition from stage 1 to stage 2 sleep was not affected by 0.8-Hz stimulation, although it revealed to be delayed with random stimulation ( $P < 0.05$ , compared with sham). Differences in the transition time from state 2 sleep into SWS were not significant ( $P = 0.78$  versus random stimulation;  $P = 0.37$  versus sham).



**Figure 1.** Mean ( $\pm$ SEM) transition times for the three stimulation conditions (0.8-Hz stimulation, random stimulation, sham) to reach sleep stage 1 (S1) from wakefulness (left), stage 2 sleep (S2) from S1 (middle), and slow-wave sleep (SWS) from S2 (right). ‘Wakefulness’ refers to the time when lights were turned off, and only transitions to S1 were considered that were followed by S2. \* $P < 0.05$ , \*\* $P < 0.01$ , for paired *t*-tests.



**Figure 2.** Mean ( $\pm$ SEM) time course (a) of spindle power (12–15 Hz, at Cz), with reference to lights off (0 min) for the 0.8-Hz stimulation condition (solid line) and for the random stimulation condition (dashed line). The black horizontal bar indicates an interval 4–8 min after lights off where spindle power differed significantly ( $P < 0.01$ ) between conditions. (b) Mean ( $\pm$ SEM) spindle power at Cz for the 0.8-Hz stimulation (white), random stimulation (grey) and sham (black) stimulation conditions 4–8 min after lights off. \*\* $P < 0.01$  for paired t-test.



**Figure 3.** Mean ( $\pm$ SEM) time course (left panels) of SO power (0.5–1 Hz, at Fz) averaged with reference to lights off (a) and with reference to the onset of sleep stage 2 (b), for the 0.8-Hz stimulation condition (solid line) and for the sham condition (dotted line). Black horizontal bars indicate intervals where SO power differed significantly ( $P < 0.01$ ) between conditions. Mean ( $\pm$ SEM) SO power for these intervals is indicated for the 0.8-Hz stimulation (white), random stimulation (grey) and sham (black) stimulation conditions in the right panels. \*\* $P < 0.01$  for paired t-test. Effects of 0.8-Hz stimulation on SWA were less consistent than those on SO activity and overall revealed only marginal significance. Effects on theta activity as well as on slow spindle activity remained non-significant. Analyses of fast

spindle power (12–15 Hz) confirmed that stimulation, and particularly 0.8-Hz stimulation, delayed the occurrence of stable NonREM sleep (Fig. 2a). A few minutes after subjects were allowed to sleep, fast spindle power started to increase. However, this increase was delayed during rhythmic 0.8-Hz stimulation. Thus, spindle power (at Cz, averaged time-locked to lights off) was reduced during 0.8-Hz stimulation, most consistently if compared with random stimulation ( $P < 0.01$ ; Fig. 2b).

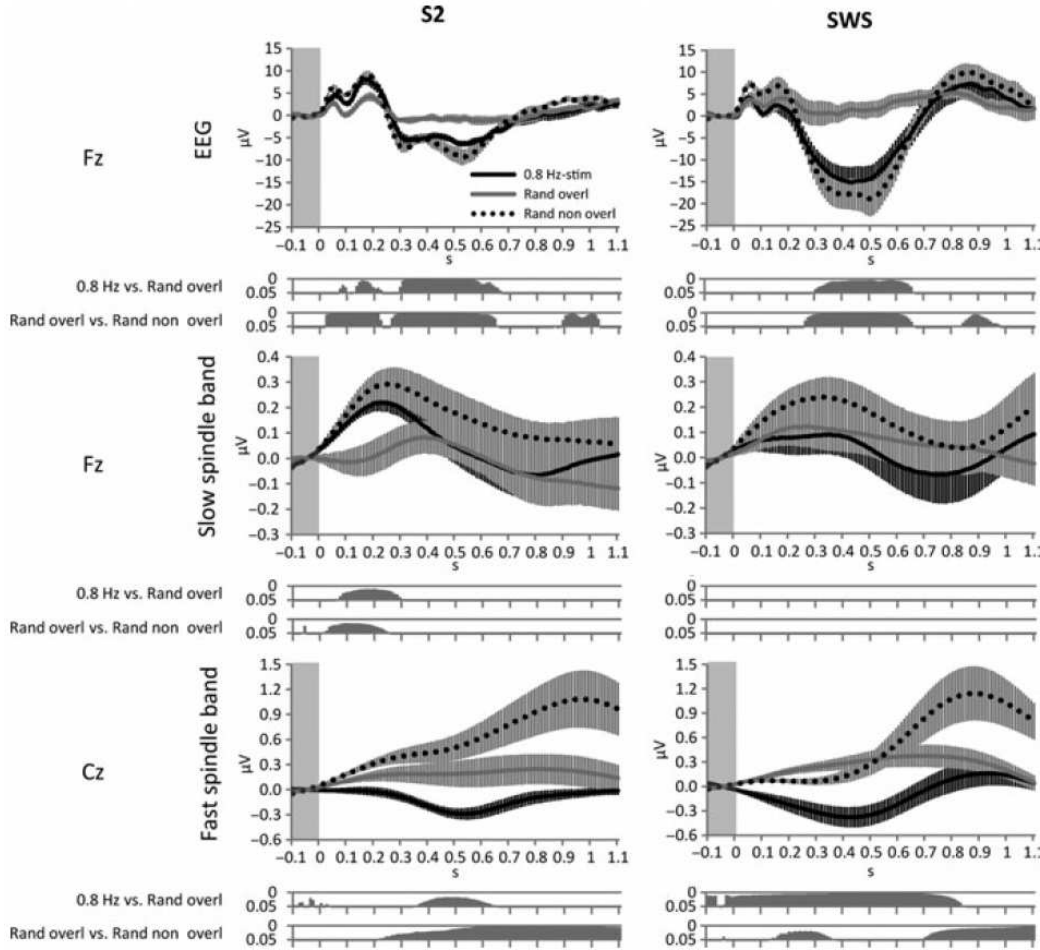
### 0.8-Hz stimulation enhances SO activity once stage S2 sleep has manifested

Contrary to our expectation, 0.8-Hz stimulation, compared with random stimulation and sham, did not affect power in the SO frequency band (0.5–1 Hz) during the waking period before lights off as well as thereafter in the beginning of the sleep period (Fig. 3a). However, the rhythmic 0.8-Hz stimulation had an impact once the subject advanced into NonREM sleep stage 2. Averaging SO power time-locked to the onset of sleep stage 2 revealed that 0.8-Hz stimulation produced a distinct increase in SO power 10–25 min later, with this effect coinciding with the occurrence of SWS (Fig. 3b). The effect was most consistently observed when compared with the sham condition ( $P = 0.011$ , for analyses with reference to lights off, Fig. 3a;  $P = 0.004$ , for analyses with reference to the onset of stage 2 sleep, Fig. 3b). Changes in sleep onset latency during the 0.8-Hz stimulation condition (with reference to sham stimulation) and SO power were not correlated ( $r = 0.266$ ,  $P = 0.458$ ), excluding that increases in SO power were an immediate consequence of the delaying effect of the stimulation on sleep onset. Effects

of 0.8-Hz stimulation on SWA were less consistent than those on SO activity and overall revealed only marginal significance. Effects on theta activity as well as on slow spindle activity remained non-significant.

### Auditory stimulation modulates slow and fast spindle activity

Averaged AEPs to the stimulation were determined separately for the 0.8-Hz stimulation and the random stimulation condition, with the responses for the random stimulation condition additionally separated to stimuli that were or were not separated by more than 1.25 s from the previous and following sound ('non-overlapping' versus 'overlapping' responses). Consistent with previous studies (e.g. Colrain and Campbell, 2007), AEPs during stage 2 sleep revealed a positive component about 200 ms post-stimulus onset followed by a double-peaked negative component 300–600 ms post-stimulus onset (Fig. 4, upper panel). The two peaks of the latter component tended to merge into a single broad hyperpolarization during SWS, which was then followed by a depolarization at 900 ms post-stimulus. In fact, during the SWS this late negative-to-positive AEP complex (300–900 ms post-stimulus) showed some similarity with a SO. Generally, the AEP potential components were smallest for the 'overlapping' responses, as compared with the 'nonoverlapping' responses and with the responses to the 0.8-Hz stimulation (see Fig. 4 for statistical comparisons), reflecting the refractoriness of the AEP with shorter ISIs (Durrant and Boston, 2006). AEPs in the random stimulation condition

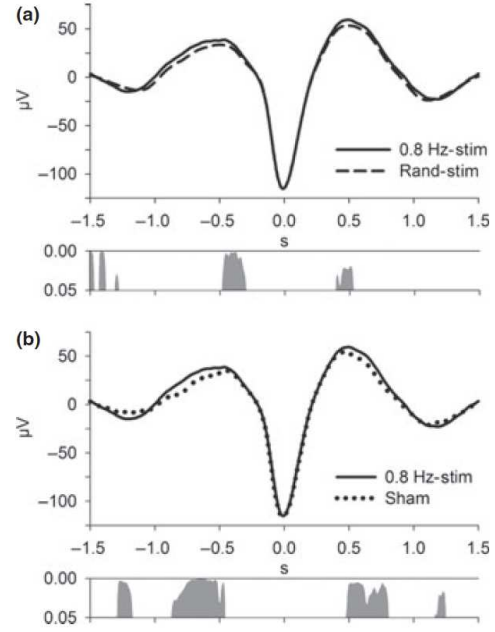


**Figure 4.** AEPs (mean  $\pm$  SEM) derived from 0.8-Hz stimulation (black line) and random stimulation conditions (grey line for overlapping and dotted line for non-overlapping stimuli) and categorized by sleep stage S2 (left column) and slow-wave sleep (SWS; right column), as well as conventional electroencephalogram (EEG) band (0.15–30 Hz, top row), slow spindle band (9–12 Hz, middle row) and fast spindle band (12–15 Hz, bottom row). Bottom lines indicate point-wise statistical comparison between the 0.8-Hz stimulation condition and the overlapping random stimulation (Rand overl), and between the overlapping and non-overlapping (Rand non-overl) stimuli of the random stimulation condition. As the non-overlapping stimuli included the lowest number of stimuli, for respective comparisons stimulus subsets of equal size were randomly drawn from the category of overlapping stimuli as well as from the set of stimuli in the 0.8-Hz stimulation condition. Vertical grey bars indicate intervals used for baseline normalization. AEPs during S2:  $n = 211.8 \pm 13.8$ ; and during SWS:  $n = 194.9 \pm 18.5$ .

were also significantly smaller than those during the 0.8-Hz stimulation condition, when AEPs were averaged across all stimuli (overlapping and non-overlapping stimuli of the random stimulation condition, and all stimuli of the 0.8-Hz stimulation condition; Fig. S1). Root mean square slow spindle activity averaged with reference to stimulus onset showed a maximum shortly before the AEP negativity 300–600 ms post-stimulus, but did not differ for the different stimulus types during SWS (overlapping, non-overlapping random stimulation, 0.8-Hz stimulation; Fig. 4). Fast spindle activity was suppressed during the AEP negativity (300–600 ms post-stimulus), particularly during the 0.8-Hz stimulation condition (see Figs 4 and S1 for statistical comparisons). This decrease in the 0.8-Hz stimulation condition was also significant when compared with responses averaged across all stimuli of the random stimulation condition (Fig. S1). Fast spindle activity was enhanced during late AEP positivity (900 ms poststimulus), in particular after non-overlapping stimuli of the random stimulation condition. This late increase in fast spindle activity was also significant when all stimuli of the random stimulation condition were compared with the 0.8-Hz stimulation condition (Fig. S1).

### SOs are modulated and entrained by 0.8-Hz stimulation

Fig. 5 depicts average SOs identified during epochs of SWS occurring in the 90-min period of stimulation and during the corresponding periods of the random stimulation and the sham conditions. Notably, the 0.8-Hz stimulation did not significantly change the number of detected SO events during the 90-min stimulation period ( $n = 779.2 \pm 124.6$  versus random stimulation  $n = 730.9 \pm 54.2$ , sham  $n = 732.0 \pm 123.5$ ,  $P > 0.661$ ), underlining that the effect of the stimulation was primarily on the temporal entrainment of SOs. Averaging was performed with reference to the negative half-wave peak of the SOs. Comparison of the SOs in the three stimulation conditions shows a significantly stronger depolarization of the positive depolarizing up states before and after the negative half-wave of a SO event for the 0.8-Hz stimulation condition, in comparison with both the random and sham conditions during intervals of greatest difference (see Fig. 5 for statistical comparisons). To analyse the occurrence of trains of SOs in the different stimulation conditions, we calculated auto-event correlation histograms that visualize the timing between successive SOs. These auto-event correlation histograms indicated that rhythmic acoustic 0.8-Hz stimulation indeed induced more regular trains of SOs during SWS (Fig. 6). This was apparent by significant ( $P < 0.05$ ) increases in the frequency of SO peaks around time points being multiples of 1.25 s during the 0.8-Hz stimulation condition, i.e. the emerging SOs adapted to the external drive. Again this entraining effect of 0.8-Hz stimulation on the SOs was significant in comparison with both the random stimulation and the sham conditions.



**Figure 5.** Mean SO (at Fz) during SWS periods of the 90-min stimulation interval for (a) the 0.8-Hz stimulation condition (solid line) and random stimulation (dashed line), and (b) 0.8-Hz stimulation and sham conditions (dotted line). Differences in the potential level are indicated by point-wise statistical comparisons positioned below each average. SEMs were generally  $< 3$   $\mu V$  and are not shown because at the selected scaling they are not discernible.

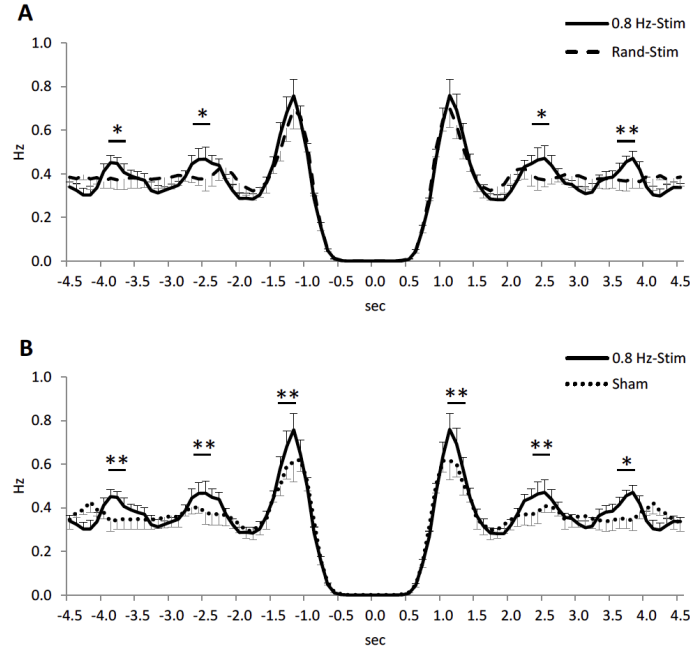
### Sleep architecture remained unchanged during and after stimulation

An analysis of the sleep stage distribution following the first 90 min was performed to examine whether continuing effects were present and affected remaining sleep. Table 1 lists sleep parameters for the 90-min period of stimulation as well as for the remaining sleep epoch, and did not indicate any difference between the stimulation conditions in time spent in the different sleep stages for both periods, i.e. during and after stimulation ( $P > 0.096$ , for all comparisons). Also, number of arousals did not significantly differ between conditions, excluding that auditory stimulation disturbed sleep.

## Discussion

Our data indicate that rhythmic acoustic stimulation with a slow 0.8-Hz frequency mimicking the frequency of natural EEG SOs does not enhance SO activity in the waking brain, but leads to a distinct delay in the onset of sleep, which was paralleled by suppression of spindle power. However, periodic 0.8-Hz stimulation increases spectral power in the SO band during NonREM and SWS, although there was no earlier onset of SWS. Averaging of AEPs and, in parallel, evoked spindle activity revealed a stimulus-induced modulation of fast spindle activity reminiscent to that during





**Figure 6.** Auto-event correlation of detected SOs (mean  $\pm$  SEM) for recordings from Fz during SWS periods of the 90-min stimulation interval, for the 0.8-Hz stimulation condition (solid lines), and (a) the random stimulation condition (dashed line) and (b) the sham condition (dotted line). Negative peaks of detected SO events were used to perform auto-event correlation analyses (for 9-s windows around negative half-wave peaks, 0.1-s bin-size). The x-axis indicates time intervals between successive SO events; the y-axis indicates the rates of SO events occurring at a given time interval. Thus, y-values represent the likelihood of a SO event occurring at a specific time before or after an identified SO event (as defined by its negative peak at '0 s'). Black bars denote 0.3-s intervals centrally positioned at multiples of 1.25 s, corresponding to the 0.8-Hz rhythm of acoustic stimulation. \* $P < 0.05$ , \*\* $P < 0.01$ , for paired t-tests. Note the first peak in the auto-event correlation histogram (at 1.25 s) is identical for both the 0.8-Hz stimulation and random stimulation conditions, reflecting that also randomly presented stimuli evoked two succeeding SOs. However, the succeeding peaks of the histogram at 2.5 and 3.75 s were significantly higher for the 0.8-Hz stimulation condition than the random stimulation condition, indicating that the 0.8-Hz stimulation induced longer trains of succeeding SOs.

**Table 1 Sleep stage distribution for different time intervals and conditions**

Parameter	Stimulation period			Remaining sleep		
	0.8-Hz Stim	Rand stim	Sham	0.8-Hz Stim	Rand stim	Sham
TST (min)	90.00 $\pm$ 0.0	89.70 $\pm$ 0.1	90.00 $\pm$ 0.0	387.90 $\pm$ 4.0	386.40 $\pm$ 4.9	391.95 $\pm$ 1.2
W (%)	4.56 $\pm$ 0.6	3.45 $\pm$ 1.4	3.56 $\pm$ 0.6	1.28 $\pm$ 0.5	0.93 $\pm$ 0.3	0.97 $\pm$ 0.3
S1 (%)	5.61 $\pm$ 0.7	5.63 $\pm$ 1.1	6.28 $\pm$ 0.8	2.92 $\pm$ 0.7	2.22 $\pm$ 0.4	1.97 $\pm$ 0.5
S2 (%)	46.56 $\pm$ 6.0	43.65 $\pm$ 3.0	50.22 $\pm$ 6.0	55.94 $\pm$ 1.8	52.61 $\pm$ 2.0	58.08 $\pm$ 2.0
SWS (%)	39.06 $\pm$ 6.1	38.01 $\pm$ 3.3	36.83 $\pm$ 5.9	10.04 $\pm$ 1.4	13.41 $\pm$ 1.3	10.56 $\pm$ 1.4
REM (%)	1.11 $\pm$ 1.1	4.47 $\pm$ 1.9	0.00 $\pm$ 0.0	23.34 $\pm$ 1.5	23.92 $\pm$ 1.3	23.04 $\pm$ 1.1
Arousals (%)	3.06 $\pm$ 0.49	4.63 $\pm$ 0.72	3.06 $\pm$ 0.56	5.87 $\pm$ 0.54	6.73 $\pm$ 0.51	5.68 $\pm$ 0.68
Mean percentage ( $\pm$ SEM) of time spent in different sleep stages for the three stimulation conditions (0.8-Hz stimulation, random stimulation, sham) and two time intervals: the 90-min stimulation period (measured from lights off) and the remaining stimulation-free sleep period (until awakening at 07:00 hours). There were no significant differences between conditions. REM, rapid eye movement; S1, sleep stage 1; S2, sleep stage 2; SWS, slow-wave sleep; TST, total sleep time; W, wake.						

spontaneous SOs specifically in the 0.8-Hz stimulation condition. Amplitude and auto-correlation analyses of SOs revealed that the 0.8-Hz stimulation not only increased the depolarizing up phase of SOs, but effectively entrained these oscillations to the 0.8-Hz rhythm of stimulation.

The effect of acoustic stimulation on the sleeping brain has been thoroughly investigated. However, the majority of these studies in humans either focused on amplitudes and latencies of specific components of the AEP response to assess information processing during sleep (Bastuji et al., 2002; Campbell and Colrain, 2002; Dang-Vu et al., 2011), or aimed at a disturbance of sleep by acoustic stimulation to suppress specific sleep stages like SWS and to investigate its consequence on, for instance, learning performances during subsequent wakefulness (Landsness et al., 2009; Van der Werf et al., 2009). In anaesthetized guinea pigs, regular

sound stimulation produced an entrainment of SO activity in thalamic neurons (Gao et al., 2009). Against this background, the present study is, to the best of our knowledge, the first to examine whether rhythmic stimulation can be used to entrain brain EEG oscillatory phenomena like the SO during sleep in humans.

In agreement with other experimental studies, our findings show that acoustic stimulation during SWS evokes a specific electrophysiological response, consisting of a strong hyperpolarization after about 500 ms followed by a depolarization, which is maximal at about 900 ms (Amzica and Steriade, 1998; Plihal et al., 1996; Riedner et al., 2011). A strong hyperpolarization followed by a depolarization is characteristic for the SOs that are detected as such by our algorithm. It is well established that this evoked response is associated with strong cortical synchronized activity and interacts with the thalamo-cortical system to generate the K-complex (Bastien and Campbell, 1994; Contreras and Steriade, 1995). K-complexes bear striking similarities with the SO in morphology and generating mechanisms, although differences may also exist between these phenomena (Amzica, 2010; Cash et al., 2009; De Gennaro et al., 2000; Riedner et al., 2011). Thus, in light of the fact that the increase in SO power during the 0.8-Hz stimulation condition was not statistically different from that of random stimulation, it could be argued that these effects on SO power were mainly driven by evoked K-complexes to the sounds rather than by an entrainment to the rhythmic 0.8-Hz stimulation. To clarify this issue, we analysed AEPs that indeed revealed that for stimuli presented during random stimulation with short ('overlapping' ISIs, the late negative-to-positive component complex bearing great similarity with the SO was significantly smaller than in the AEP to the stimuli presented at 0.8 Hz, likely reflecting the refractoriness of the AEP response with short ISIs (Durrant and Boston, 2006). Also, AEPs averaged across all sounds of the random stimulation condition revealed on average smaller component amplitudes than in the 0.8-Hz stimulation condition, especially during sleep stage 2. However, parallel analysis of evoked fast spindle activity revealed that the observed increase in SO activity during the 0.8-Hz stimulation condition cannot be entirely reduced to K-complexes (evoked at this specific ISI). K-complexes are typically associated with a transitory increase in fast spindle activity (Contreras and Steriade, 1995). In fact, such an increase was observed in response to the sounds (about 900 ms post-stimulus) during random stimulation, with this increase significantly exceeding that during 0.8-Hz stimulation. By contrast, in the 0.8-Hz stimulation condition the suppression of fast spindle activity during the preceding hyperpolarization of the AEP (300–600 ms post-stimulus) predominated (Figs 4 and S1). Thus, whereas isolated random stimuli caused a steady increase in fast spindle activity over the entire 1.1-s post-stimulus interval, the sounds of the 0.8-Hz stimulation condition produced a phase-dependent modulation of fast spindle activity quite similar to that observed during spontaneous SOs

(Mölle and Born, 2011; Mölle et al., 2002). Moreover, similar to the temporal pattern during spontaneous SOs, also slow spindle activity during the 0.8-Hz stimulation condition (during stage 2 sleep) was significantly increased at the transition of the AEP into the negative phase ( $\sim 300$  ms post-stimulus; Mölle et al., 2011). This differential patterning of fast and slow spindle activity, which is specifically observed during the 0.8-Hz stimulation condition and which closely mimics the temporal relationships between slow and fast spindles during spontaneous SOs, strongly argues for the view that factors other than K-complexes significantly contribute to the entrainment of SO activity observed during 0.8-Hz stimulation. The effect on SO amplitude per se being only of moderate size might reflect habituation concurrently developing with the periodic signal presentation. In demonstrating that rhythmic acoustic stimulation can induce and entrain sleep SOs, our data suggest the use of this approach in the study of functions known to be promoted by SOs, such as the consolidation of memory and the post-sleep facilitation of encoding of new memories (D Antonenko, S Diekelmann, C Olsen, J Born and M Mölle, 2012, submitted; Marshall et al., 2006; Van der Werf et al., 2009). Such studies may reveal the induction of trains of SOs to be more critical for memory processes than mere changes in SO amplitude (Mölle et al., 2011).

A main finding of our study is that the efficacy of 0.8-Hz stimulation is state dependent. SO power was enhanced by the rhythmic stimulation only after stable NonREM sleep stage 2 had become manifest. No similar effects were obtained during waking before sleep, and the tone stimulation also did not shorten sleep latency, which diverges from a previous study (Bohlin, 1971), although that study used much longer ISIs (varying between 20 and 40 s). A comparable dependency of the effects of tone stimulation on the brain state has been shown for the AEP showing characteristic changes in its waveform (and frequency content) when the brain transits from wakefulness to light sleep and SWS (Campbell and Colrain, 2002; Cote, 2002). Here we revealed a brain state dependence specifically in terms of predominant EEG rhythm. The failure of 0.8-Hz stimulation to increase SO activity in the waking brain, when the EEG is dominated by faster frequencies, together with significant delay of sleep onset resulting from the periodic 0.8-Hz stimulation implies that the brain's susceptibility to an external drive is highly sensitive to its current state of vigilance. Wakefulness either does not allow an entrainment per se, or this specific brain state is characterized by a resonance frequency disjoint to the 0.8-Hz stimulation, which explains the delayed transition into sleep stage 1 as the system's dynamics are perturbed. The idea of a resonance effect induced by 0.8-Hz stimulation is in particular coherent with the finding of higher accumulation of SO power after SWS was reached. However, although suggesting a brain state dependency of the stimulation effect, our data do not entirely rule out that other factors, like circadian rhythm, added to the effects of 0.8-Hz stimulation on EEG activity occurring selectively during sleep.



The state dependence of the effects of 0.8-Hz stimulation on SO activity reported here has been similarly observed in recent studies using tDCS oscillating at a frequency of 0.75 Hz. The oscillating tDCS induced wide-spread endogenous SO activity when applied during NonREM and SWS, whereas the increase in SO activity was marginal and locally restricted to the prefrontal cortex when the stimulation was applied to the waking brain (Kirov et al., 2009; Marshall et al., 2006). However, the waking brain responded with an increased theta activity to 0.75-Hz tDCS. The convergence of these findings tempts to speculate about the concept of a resonance frequency that characterizes the oscillatory EEG response to rhythmic stimulation for the different brain states. In this view, SWS is essentially characterized by a 0.8-Hz resonance frequency, whereas wakefulness as well as light sleep at the transition to deep sleep represent brain states resonating at frequencies different from the 0.8-Hz stimulation frequency applied here. The modulation of 12–15-Hz spindle activity caused by the random stimulation suggests for the transitory period of light sleep a faster resonance frequency above the SO range, as the random stimulation contained a high proportion of shorter ISIs. Consistent with this view, in a previous study, instrumental conditioning of sensorimotor rhythm in the 12–15-Hz frequency band effectively decreased sleep onset latency (Hoedlmoser et al., 2008). Yet, such assumptions are in need of experimental validation. In conclusion, our results indicate that rhythmic acoustic stimulation can be used to induce sleep SO activity, which makes it indeed a promising and simple approach for the investigation of putative sleep functions linked to the SO rhythm.

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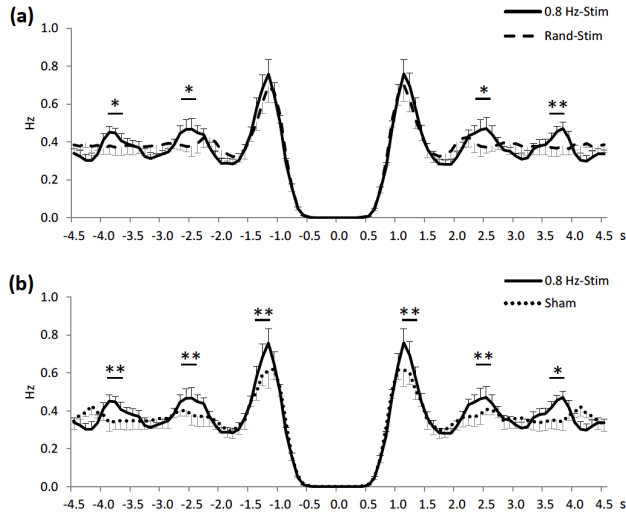
**Conflict of Interest** None of the authors has any conflict of interest. We confirm that we are in compliance with the Journal Sleep Research policy.

## References

- [1] Achermann, P. and Borbely, A. A. Low-frequency (<1 Hz) oscillations in the human sleep electroencephalogram. *Neuroscience*, 1997, 81: 213–222.
- [2] Amzica, F. Comment on ‘The human K-complex represents an isolated cortical down-state’. *Science*, 2010, 330: 35.
- [3] Amzica, F. and Steriade, M. Cellular substrates and laminar profile of sleep K-complex. *Neuroscience*, 1998, 82: 671–686.
- [4] Bastien, C. and Campbell, K. Effects of rate of tone-pip stimulation on the evoked K-complex. *J. Sleep Res.*, 1994, 3: 65–72.
- [5] Bastuji, H., Perrin, F. and Garcia-Larrea, L. Semantic analysis of auditory input during sleep: studies with event related potentials. *Int. J. Psychophysiol.*, 2002, 46: 243–255.
- [6] Bohlin, G. Monotonous stimulation, sleep onset and habituation of the orienting reaction. *Electroencephalogr. Clin. Neurophysiol.*, 1971, 31: 593–601.
- [7] Campbell, K. B. and Colrain, I. M. Event-related potential measures of the inhibition of information processing: II. The sleep onset period. *Int. J. Psychophysiol.*, 2002, 46: 197–214.
- [8] Cash, S. S., Halgren, E., Dehghani, N. et al. The human K-complex represents an isolated cortical down-state. *Science*, 2009, 324: 1084–1087.
- [9] Colrain, I. M. and Campbell, K. B. The use of evoked potentials in sleep research. *Sleep Med. Rev.*, 2007, 11: 277–293.
- [10] Contreras, D. and Steriade, M. Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J. Neurosci.*, 1995, 15: 604–622.
- [11] Cote, K. A. Probing awareness during sleep with the auditory oddball paradigm. *Int. J. Psychophysiol.*, 2002, 46: 227–241.
- [12] Dang-Vu, T. T., Bonjean, M., Schabus, M. et al. Interplay between spontaneous and induced brain activity during human non-rapid eye movement sleep. *Proc. Natl Acad. Sci. USA*, 2011, 108: 15438–15443.
- [13] De Gennaro, L., Ferrara, M. and Bertini, M. The spontaneous Kcomplex during stage 2 sleep: is it the ‘forerunner’ of delta waves? *Neurosci. Lett.*, 2000, 291: 41–43.
- [14] Diekelmann, S. and Born, J. The memory function of sleep. *Nat. Rev. Neurosci.*, 2010, 11: 114–126.
- [15] Durrant, J. D. and Boston, J. R. Stimuli for auditory evoked potential assessment. In: R. F. Burkhard, M. Don and J. J. Eggermont (Eds) *Auditory Evoked Potentials: Basic Principles and Clinical Application*. Lippincott Williams and Wilkins, Philadelphia, 2006: 42–72.
- [16] Gao, L., Meng, X., Ye, C. et al. Entrainment of slow oscillations of auditory thalamic neurons by repetitive sound stimuli. *J. Neurosci.*, 2009, 29: 6013–6021.
- [17] Hoedlmoser, K., Pecherstorfer, T., Gruber, G. et al. Instrumental conditioning of human sensorimotor rhythm (12–15 Hz) and its impact on sleep as well as declarative learning. *Sleep*, 2008, 31: 1401–1408.
- [18] Kirov, R., Weiss, C., Siebner, H. R., Born, J. and Marshall, L. Slow oscillation electrical brain stimulation during waking promotes EEG theta activity and memory encoding. *Proc. Natl Acad. Sci. USA*, 2009, 106: 15460–15465.
- [19] Landsness, E. C., Crupi, D., Hulse, B. K. et al. Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. *Sleep*, 2009, 32: 1273–1284.
- [20] Marshall, L., Helgadottir, H., Mölle, M. and Born, J. Boosting slow oscillations during sleep potentiates memory. *Nature*, 2006, 444: 610–613.
- [21] Massimini, M., Ferrarelli, F., Esser, S. K. et al. Triggering sleep slow waves by transcranial magnetic stimulation. *Proc. Natl Acad. Sci. USA*, 2007, 104: 8496–8501.
- [22] Mölle, M. and Born, J. Slow oscillations orchestrating fast oscillations and memory consolidation. *Prog. Brain Res.*, 2011, 193: 93–110.
- [23] Mölle, M., Marshall, L., Gais, S. and Born, J. Grouping of spindle activity during slow oscillations in human non-rapid eye movement sleep. *J. Neurosci.*, 2002, 22: 10941–10947.
- [24] Mölle, M., Bergmann, T. O., Marshall, L. and Born, J. Fast and slow spindles during the sleep slow oscillation: disparate coalescence and engagement in memory processing. *Sleep*, 2011, 34: 1411–1421.
- [25] Plihal, W., Weaver, S., Mölle, M., Fehm, H.L. and Born, J. Sensory processing during early and late nocturnal sleep. *Electroencephalogr. Clin. Neurophysiol.*, 1996, 99: 247–256.
- [26] Rattenborg, N. C., Martinez-Gonzalez, D., Roth, T. C. and Pravosudov, V. V. Hippocampal memory consolidation during sleep: a comparison of mammals and birds. *Biol. Rev. Camb. Philos. Soc.*, 2011, 86: 658–691.

- [27] Rechtschaffen, A. and Kales, A. A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. Gov. Printing Office, Washington DC, 1968.
- [28] Riedner, B. A., Hulse, B. K., Murphy, M. J., Ferrarelli, F. and Tononi, G. Temporal dynamics of cortical sources underlying spontaneous and peripherally evoked slow waves. *Prog. Brain Res.*, 2011, 193: 201–218.
- [29] Sanchez-Vives, M. V. and McCormick, D. A. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosci.*, 2000, 3: 1027–1034.
- [30] Steriade, M. Grouping of brain rhythms in corticothalamic systems. *Neuroscience*, 2006, 137: 1087–1106.
- [31] Steriade, M., Nunez, A. and Amzica, F. A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J. Neurosci.*, 1993, 13: 3252–3265.
- [32] Tononi, G. and Cirelli, C. Sleep function and synaptic homeostasis. *Sleep Med. Rev.*, 2006, 10: 49–62.
- [33] Van der Werf, Y. D., Altena, E., Schoonheim, M. M. et al. Sleep benefits subsequent hippocampal functioning. *Nat. Neurosci.*, 2009, 12: 122–123.
- [34] Vyazovskiy, V. V., Faraguna, U., Cirelli, C. and Tononi, G. Triggering slow waves during NREM sleep in the rat by intracortical electrical stimulation: effects of sleep/wake history and background activity. *J. Neurophysiol.*, 2009, 101: 1921–1931.

## Supporting Information



**Figure S1.** AEPs (mean  $\pm$  SEM) derived from all auditory stimuli during 0.8-Hz stimulation (black line) and random stimulation conditions (grey line), and categorized by sleep stage S2 (left column) and SWS (right column), as well as conventional EEG band (0.15–30 Hz, top row), slow spindle band (9–12 Hz, middle row) and fast spindle band (12–15 Hz, bottom row). Additionally, point-wise statistical comparisons are indicated below each AEP. Vertical grey bars indicate intervals for baseline normalization. AEPs during S2 for 0.8-Hz stimulation:  $2001.5 \pm 254.5$  and random stimulation:  $1904.4 \pm 130.0$ ; and during SWS for 0.8-Hz stimulation:  $1685.0 \pm 261.4$  and random stimulation:  $1593.8 \pm 138.7$ .